

To the editor:

We thank the reviewers for the recognition of the interest of our study and the time they spent in reviewing this collective work. In the original version we tried to find the best compromise between presenting a large data set (including physicochemical parameters and the whole microbial food web) and a paper which could stand independently from the other papers of the special volume. In the revised version, we took seriously into consideration all the reviewer's comments, but we were also mindful to keep a reasonable balance between the many different parameters presented and not to extend the length of the text, figures and tables beyond acceptable levels, or to add details already or soon to be available in other papers in the same special issue.

Below please find a detailed response to all of the comments, point-by-point and a description of corresponding changes made in the manuscript. In the revised version all the changes are highlighted in colour to facilitate locating the new text.

The last part of the discussion (4.2) is entirely reorganised.

We trust that you will find that the revised version fully accounts for the comments and concerns of the reviewers and we look forward to hearing from you soon.

For all the authors

Urania Christaki

Blue- The reviewers comments

Black- Our "Author Comments"

Red- Text added to the manuscript in response to the comments of the reviewers

Issues raised by both reviewers 1 and 2

A. There is little evidence that A, B and C stations were indeed eddies and consequently: whatever is said in the paper lacks credibility.

Response

We gave a general description of the eddies (please see part 3.2 and Fig. 3, of the original 'discussion' version). In this and the other papers in this special volume we refer to the introductory paper for a complete description (Moutin et al., Introductory paper to special issue) which will give details of the sampling strategy and the physical environment.

However, to satisfy the objections of reviewers 1 and 2 we now provide more information (see below) in our paper and below.

A. Locating and tracking mesoscale eddies & distance between stations

A brief description of the strategy employed in choosing stations, identifying, tracking and sampling eddies is now included. It appears in section 2.1 of the revised version. Please also note that the figure of salinity is replaced by a new figure (2) of salinity and nitrates. More explanations and clear reference to the BOUM special issue will be given in the introductory paper by Moutin et al. are given also below:

TEXT FROM SECTION 2.1, paragraph 2

Biological data presented in this study are based on surface-layer sampling (8-10 depths from 0 to 200 m) of 14 representative 'short duration stations' and the 3 'long duration sites' situated in the centre of anticyclonic eddies. The approximate location of the gyres were determined using satellite imagery and the forecast from MERCATOR. The exact locations of the eddies were determined on board from a rapid (12 h) high resolution survey using XBT, thermosalinograph and ADCP data to precisely locate sites with low potential advection. At each site, before starting the 96 h process study, a 24h physical and chemical characterisation of the area was carried out. The area was surveyed by the exploration of a grid consisting of 16 sub-stations in a 9x9 miles geographic area centered around the site. Data for each sub-station were obtained from 0 to 500 m or 1000 m depth with CTD casts. The salinity and nitrate profile (Fig. 2a-c) are here presented to show the "halostad" and "nutrientstad" (large zones in depth of constant salinity or nitrate which characterize the core depth of the eddies). These 'stad' correspond also to low variations with depth in density, oxygen and temperature as reported by Moutin et al., (in preparation, this issue). The 2 eddies B and C in Eastern Mediterranean exhibit deep cores constituted by Levantine Intermediate water, of higher salinity while the core of the eddy A was formed with Surface Modified Atlantic water of lower salinity than outside the eddy. For all eddies the core density is lower and the temperature and oxygen are higher than outside the eddies at the same depths.

TEXT FROM SECTION 3.2

Sites A, B and C were located in the centres of 3 distinct anticyclonic eddies across the trophic gradient. The physical data indicated that the 3 eddies were all at least several months old and that the process study stations were made in the centre of the eddies. The salinity and nitrate profiles, used to characterise the halostads and nutrientstads (large zones in depth of constant salinity and nitrate), allowed estimation of core depth of each eddy (Fig. 2a-c, for details see in preparation, Moutin et al., this issue).

We would like to point out that the Moutin et al. 'introductory paper' of the BOUM special issue which will include a full description of the eddies, by choice of the editors and organisers, shall be the last to be submitted for the special issue. If judged absolutely necessary, we can provide an annex for the revised version which would include the material such as that below, presently in preparation as part of the 'introductory paper'.

EXAMPLES OF POSSIBLE ANNEXE MATERIAL to the revised version:

On Figure 9 (Moutin et al.), the top row panels show T S and AOU vertical profiles at each eddy centre (bold) and outside (thin), and the bottom row nitrate (from bottles) and density profiles with other variables not discussed here. The depth range of horizontal difference in - out induced by core of each eddy is clear and halostad with strong differences in AOU and NO₃ are best marked than thermostad and pycnostad. It a reason why we retain a simplified version (Figure 4 of the in discussion article, Saliniy and Nitrate) of the full information presented in Moutin et al.. According to these observations, the eddy age is greater than 4 months old.

Eddy C exhibits water more saline in its core, which is mostly constituted by LIW from the Ionian sea, more ventilated (lower AOU content, Moutin et al. fig. 9) and a lower nitrate content, indicating a better vertical mixing within the eddy core.

Eddy B is more complex because in fact two halostads were found one between 250 and 600 dbars and a small near 100 -150 dbars less saline which was weakly evident in AOU due to proximity with the euphotic layer. The main core is constituted by LIW which marks the Ionienne Sea salinity maximum. This feature is the signature of a thick eddy formed and deeply ventilated (-600 m) from a winter before 2008, which can only exist in the northern Eastern part of the Ionian Sea along the continental coast. Then the eddy drifted slowly towards the observation area, but for 2008 winter it was recovered by modified Atlantic water and the upper part of eddy was ventilated more deeply than surroundings and formed a additional pycnostad, halostad, thermostad, constituted of less salt water and less nutrients.

Eddy A exhibits a less saline core, mostly formed from MAW waters, more ventilated water (lower AOU content) and with lower nitrate content, indicating a better vertical mixing within the eddy core.

EXAMPLES OF POSSIBLE ANNEXE MATERIAL to the revised version:

On Figure 9 (Moutin et al.), the top row panels show T (black), S (red) and AOU (blue) vertical profiles at each eddy centre (bold) and outside (thin), and the bottom row nitrate (from bottles, cyan) and density (black) in and out profiles with other variables at eddy center

not discussed here, namely Total Chla (*10) from CTD profiles continuous green line and from bottle date (green dots) in mg/m³ and (red dots) all the in situ values of Primary Production- mgC. m⁻³.d⁻¹- obtained for long duration station. The depth range of horizontal difference in - out induced by core of each eddy is clear and halostad with strong differences in AOU and NO₃ are best marked than thermostad and pycnostad. This is one reason why we retain a simplified version (Figure 2 of the present article), but complete information will be presented in Moutin et al). According to these observations, the eddy ages are greater than or equal to 4 months old, 4 months corresponding to the elapsed time since the more recent ventilation of cores.

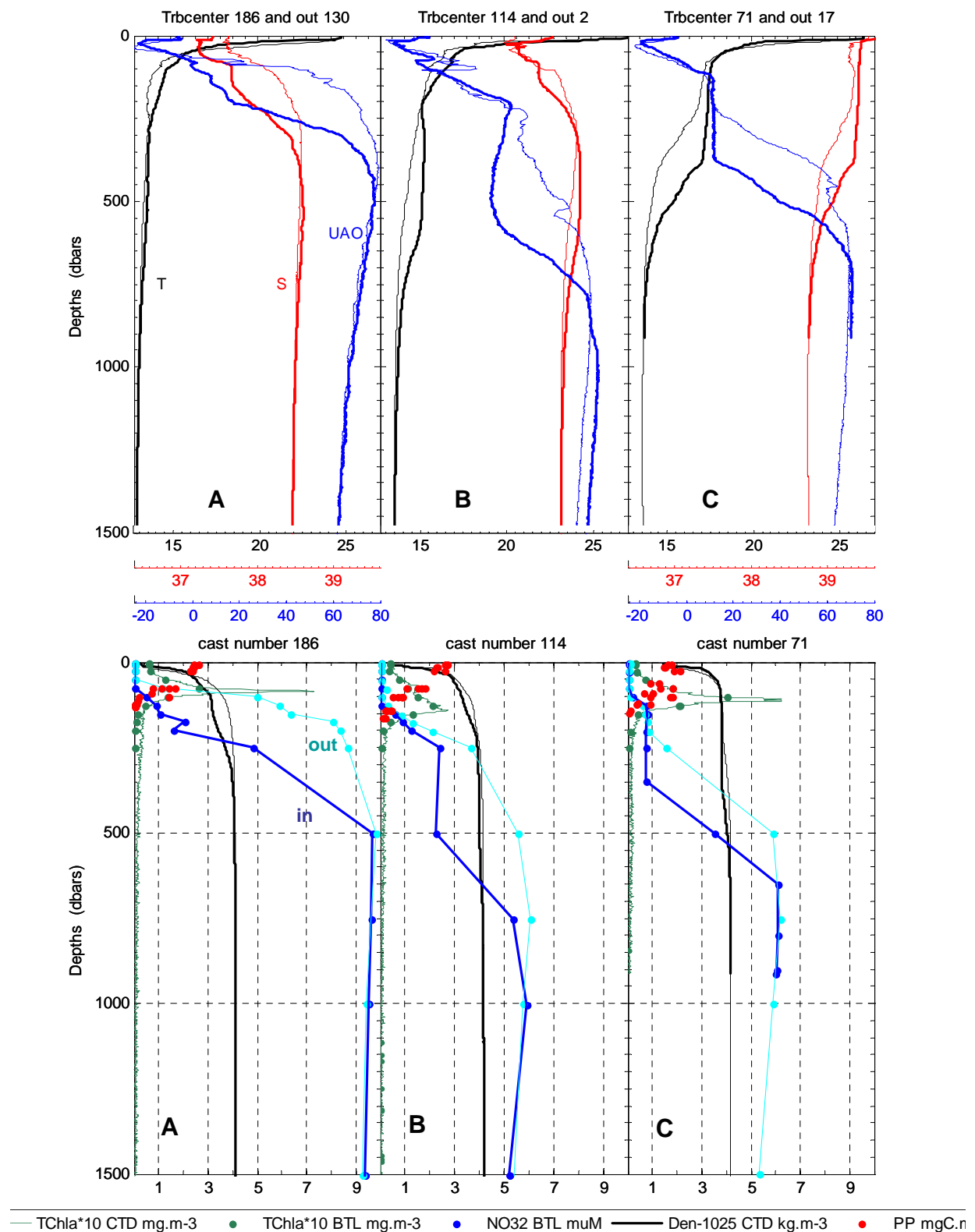


Figure 9. Primary production (P : $\text{mgC m}^{-3} \text{d}^{-1}$), chlorophyll a * 10 (Chl : $\mu\text{g l}^{-1}$), nitrate 10 concentration ($+ : \mu\text{M}$) and $\sigma_{\theta} - 25$ ($- : \text{kg m}^{-3}$), along pressure (dbar) at the 3 long duration 11 stations. BOUM cruise (June-July 2008).

C. The title misrepresents the paper.

The title and consequently the whole ‘vision’ of the paper has been re-scaled. The paper is now entitled:

Microbial food webs and metabolic state across oligotrophic waters of the Mediterranean Sea during summer.

The last part of the introduction (objectives) has been modified and accordingly other parts of the discussion. The goals have been re-formulated in the last paragraph of the Introduction:

Our study was conducted within the framework of the BOUM cruise (Biogeochemistry from Oligotrophic to the Ultra-oligotrophic Mediterranean) in summer 2008. The first objective was a complete description of the microbial food web, and in particular the heterotrophic components, along a W- E transect of 17 stations of the Mediterranean Sea during the period of summer water-column stratification. Our second objective was to estimate rates of the production and fate of organic matter, in 3 geographically distant oligotrophic environments located at the centre of anticyclonic eddies. The cores of persistent eddies are relatively isolated from surrounding waters thus these sites provide possibilities for the estimation of biogeochemical fluxes. Located along the W - E transect, we expected that these three eddies would differ not only in terms of biomass and production compared to outside reference stations located in the same basin, but also among each other. The major biogeochemical and biological parameters reported in this study are microbial stocks (from viruses to ciliates) and heterotrophic prokaryotic production at all stations while primary production and oxygen fluxes (community production and respiration) were measured only in the three eddy sites.

REVIEWER 1 COMMENTS

Review of bg-2010-358-discussions (Christaki et al. 2011, The impact of anticyclonic mesoscale structures on microbial food webs in the Mediterranean Sea)

General comments

(1) title

This manuscript describes several microbial related variables (mainly heterotrophic) along a longitudinal transect in the Mediterranean. As such it is an interesting study since a series of parameters that are not so often analyzed in oceanographic cruises and are crucial to understanding the microbial food web are provided under the same methodological conditions and within a relatively short time frame. The title promises that the study focuses on getting an understanding of the impact of anticyclonic eddies on microbial food webs. However, as one reads the introduction and the rest of the manuscript, this issue is secondary while the first

objective is clearly stated to give a *general description of the vertical and spatial distribution* of the parameters studied. The second objective is to characterize the functioning of the microbial food web inside 3 anticyclonic eddies.

As far as the eddies go, they are not properly characterized, at least with the data shown in this paper (Fig. 3, which by the way misses the T profile that the legend promises). Stations are too far apart to properly characterize the eddies and their boundaries. Also, with my limited knowledge on the subject, it would seem to me (from salinity of Fig. 3) that a clear signal is only observed in A. It is possible that the BOUM cruise had more stations if the station numbering is correlative, but even doubling the stations results in a sort of poor spatial resolution, to make sure one is measuring the biogeochemical parameters well inside an eddy. In any case, if studying the impact of the eddies is an objective, these eddies should be better defined.

Please see response to issues raised by both reviewers 1 & 2 above

(3) longitudinal trends and station 25 and 27

The results show some clear longitudinal trends, albeit with quite some noise. I would certainly discard from the general analysis stations 25 and 27; they hardly add to the longitudinal component (25) and they seem to be clearly highly influenced by land proximity (27). The authors do not need them for the longitudinal analyses and only add noise.

| The station 25 is an open water station well beyond the continental shelf (2267 m depth). The station 27 is on the continental shelf (100 m depth). Both stations showed typical oligotrophic values of W Med stations and rather than "add noise" follow nicely the E-W gradient (see fig. 2, table 1). Note also that station 17 in the Sicily straight is also over the continental slope (116 m depth, cf fig. 2). For this reason we now make clear in the paper that **"except St. 27 and 17 that were on the continental slope, the rest of our stations were situated in the open Sea"** (Section 2.1)

Note that we also added the bottom depths of the stations on Table 1.

I did the exercise of plotting the DCM versus longitude (without st 25 and 27) and a nice line results (left plot below). It is true that A and B are above the line, while C is right on the line. If we take A, B, and C out (right plot below), the change in the line is not large but variability is reduced quite a bit. The slope also increases slightly mainly owing to the removal of A. So, the authors may have a point about the eddies, if they can show that A and B were in the middle of anticyclonic eddies. C is a different story. When we take a look at biological and biogeochemical flux data (the 3 "eddies" behave differently), there is quite a bit of variability between eddies. So, overall there is no general trend, no single direction for a possible effect of the "eddies" that one can invoke to explain the variability in microbial food web dynamics. I am not saying there may not be one, just that the data is too sketchy and partial or maybe time-dependent to ascertain that there is. Regarding the flux assessments and the ecosystem balance it is a pity that such data is not available "outside" the "eddies", and thus not much can be said about the effect of eddies other than it seems to be different for all 3.

Marked longitudinal gradients in DCM depth, along with nutrient and chlorophyll concentrations are very basic, well-known, features of the Mediterranean Sea. The eddy studies were conducted with the expectation that eddies located along the gradients might show differences among each other. Comparing fluxes inside to outside the eddies was not the goal of the cruise program and would have required long-duration stations outside the eddies. These points are now hopefully clearer in the introduction.

Our study was conducted within the framework of the BOUM cruise (Biogeochemistry from Oligotrophic to the Ultra-oligotrophic Mediterranean) in summer 2008. The first objective was a complete description of the microbial food web, and in particular the heterotrophic components, along a W- E transect of 17 stations of the Mediterranean Sea during the period of summer water-column stratification. Our second objective was to estimate rates of the production and fate of organic matter, in 3 geographically distant oligotrophic environments located at the centre of anticyclonic eddies. The cores of persistent eddies are relatively isolated from surrounding waters thus these sites provide possibilities for the estimation of biogeochemical fluxes. Located along the W - E transect, we expected that these three eddies would differ not only in terms of biomass and production compared to outside reference stations located in the same basin, but also among each other. The major biogeochemical and biological parameters reported in this study are microbial stocks (from viruses to ciliates) and heterotrophic prokaryotic production at all stations while primary production and oxygen fluxes (community production and respiration) were measured only in the three eddy sites.

See also discussion part 4.2 (**note that part 4.2 was reorganised**)

A major aim of the BOUM cruise was to estimate biogeochemical fluxes in open Mediterranean waters during the period of summer water-column stratification. The biogeochemical and the stoichiometric characterisation of stations along the entire cruise transect showed the expected oligotrophic gradient from the western basin to the Levantine basins (Pujo-Pay et al, 2011). In our study we focussed on 3 well established anticyclonic eddies located within the west to east gradient. The centre of established anti-cyclonic eddies are known to be zones of nutrient depletion with low rates of biological activity compared to surrounding areas (e.g. Mourino-Carballido 2009). We found that the 3 eddies were indeed associated with low values for different metrics of the heterotrophic compartments of the microbial food web compared to the stations located outside the eddies. Metazooplankton biomass was also found to be lower inside the eddies compared to stations outside the eddies (Nowaczyk et al., 2011, this issue). We hypothesized that the general W- E gradient of oligotrophy would be detectable among the eddies. While the W-E gradient among the eddies was generally recognizable in terms of heterotrophic biomass (Fig. 5), the same was not true for fluxes. Means of integrated PPp values were higher at site B (~190 mg C m⁻² d⁻¹) and about 15 % lower at sites A and C (~160 mg C m⁻² d⁻¹). Surface oxygen production roughly

balanced by respiration. NCP was not significantly different from zero in all three sites (4 ± 15 mmol O₂ m⁻² d⁻¹) (Fig. 9) showing systems in metabolic balance (Williams, 1993).

Specific comments

Page 189, 2nd paragraph. The importance of the objectives is not in agreement with the previous paragraph and the title of the paper.

See response to both reviewers 1 and 2 above. Both parts are now changed.

Page 198, line 5. Again the first objective is to document the vertical and spatial distribution of the heterotrophic components of the microbial food web... not the effect of eddies.

This part refers to the general longitudinal features. The processes in the eddies are discussed in detail in the next section (4.2). As explained above, we followed the reviewer's suggestion in the first place. The 'effect' or 'impact of eddies' are now excluded from the revised version. We now discuss only stocks and biological fluxes into 3 major oligotrophic Mediterranean eddies situated in 3 distinct basins.

Page 190, what was the detection limit of the autoanalyzer? for nitrate? phosphate? 0.02 μmol L⁻¹. How many samples were this low?

Samples for nitrate (NO₃), nitrite (NO₂) and phosphate (PO₄) were directly collected from the Niskin bottles in 20 ml acid washed polyethylene vials. They were immediately analysed on board according to classical methods using the automated colorimetric technique (Tréguer and Le Corre, 1975; Wood et al., 1967) on a segmented flow Bran Luebbe autoanalyser II. Precision of measurements was 0.02 μM, 0.005 μM and 0.005 μM for NO₃, NO₂, and PO₄ respectively and detection limits for the procedures were 0.02 μM, 0.01 μM and 0.01 μM for NO₃, NO₂, and PO₄ respectively. Nutrient standardisation and data quality were assured through successful and continuous participation in international intercalibration exercises. During the cruise, measurements were further verified with the use of OSIL (Ocean Scientific International Ltd) marine nutrient standards (ISO 1 9001 accredited).

According to our files for the 346 samples analysed :

Phosphate = 92 samples < 0.01 μM

Nitrites = 184 samples < 0.02 μM

Nitrate = 66 samples < 0.1 μM

The following is added in the revised version, last paragraph section 2.1:

"Precision of measurements was 0.02 μM, 0.005 μM and 0.005 μM for NO₃, NO₂, and PO₄ respectively and detection limits for the procedures were 0.02 μM, 0.01 μM and 0.01 μM for NO₃, NO₂, and PO₄ respectively. Full details on nutrients are given in Pujó-Pay et al., 2011)".

Page 190, line 20. A fixed C cell⁻¹ conversion factor is crude, especially since bacterial volume information can be obtained from the FC and then a C vol⁻¹ conversion factor could be used. Since the two subpopulations (HNA and LNA) can be hypothesized to change in importance based on the hypothesis of activity differences, not using a fixed C/cell conversion factor could become critical. The authors could report an annexed table with mean SSC and FL1 values both for the different bacterial subpopulation and the corresponding beads used as internal standard, so that readers can make their own bacterial volume estimations.

Page 191, line 17, duplicate samples are hardly enough for leucine incubations. Need at least 3 or 4 replicates, and 2 killed controls.

Page 191, line 22, what is the validity of using a fixed conversion factor of 1.5 kg C mol⁻¹ leucine in the open sea?

SSC and FL1 of HNA and LNA (relative to beads) are described in a companion study (Van Wambeke et al. 2010) where their relationships with environmental variables are described. The mean relative SSC of HNA (0.0134 ± 0.0027) is only 1.3 times higher than the mean relative SSC of LNA (0.0101 ± 0.0019) in the 0-150 m layer. Furthermore, the percentage of HNA varied to a low range (25% to 58% for 0-150m layers). Thus, the mean SSC for the whole bacterioplankton population ranged 0.009 to 0.0217 for the 0-150 m layer, i.e. varied within a 2.4 fold range. This range is lower than the range of leucine incorporation rates (0.9 to 43.9 ngCl-1h-1 for 0-150 m layers, i.e; a 49-fold range), based on a constant carbon-leucine conversion factor.

The reviewer is correct in saying that biovolume has been derived from SSC signal in some work previously (Bouvier et al 2001, Felip et al 2007). However, it is also known to be far from straightforward due to the multiple factors that can influence the SSC signal. Indeed, as stated by Bouvier et al (2007) : 'SSC is related to morphological characteristics of the cells, which are not only restricted to size and may include internal granulosity, cytoskeletal proteins, cell membrane thickness and development of mucilage and other outer structures'. These concerns explain why SSC estimates of biovolume are not commonly reported and difficult to compare among studies.

Based on experimental filtration-dilution cultures, it has been shown that, the experimental conversion factor (eCF) can vary spatially and temporally: 0.49 to 1.92 kgC mole Leu⁻¹ in the Bay of Biscay (Moran & Calvo-Diaz, 2009), 0.29 to 3.25 kgC mole Leu⁻¹ in a coastal open sea transect in NW Mediterranean (Pedros Alio et al 1999), 0.98 to 3.52 kgC mole Leu⁻¹ in coastal Mediterranean (Bay of Blanes, Alonso-Saez et al, 2008). Del Giorgio et al (2011) obtained a range of 0.5 to 2.5 kgC mole Leu⁻¹ in northeastern Pacific (del Giorgio et al 2011) with higher CF at shelf, and decreasing values toward open sea stations. We think however, that the biases introduced by filtration-dilution cultures can be very important in open, oligotrophic seas (long lag phase, change of communities, confinement effect, absence of attached bacteria in the inoculum, nutrient-limitation of heterotrophic prokaryotes) leading to large upshifts of leucine incorporation rates toward the end of filtration-dilution cultures compared to those of abundances or biovolumes and thus the cumulative method used to compute eCF in filtration-dilution cultures gives low eCF. Use of these factors provides more problems than solutions (see del Giorgio et al., 2011) and we preferred using the semi-theoretical approach.

The leucine -carbon conversion factor can be calculated using a semi-theroretical approach which does not need a biovolume estimation and avoids the biases of eCFs. This protocol is based on Simon and Azam (1989), using the assumption of a constant ratio of leucine to protein and carbon biomass to protein, and is fluctuating only due to a risk of isotopic dilution (Kirchman et al, 1993; Pedros Alio et al 1999). We checked during the BOUM cruise that the dilution factor was close to 1 when using 20 nM leucine as final concentration. Hence the dilution factor was stable and equal to 1 and this is why we used a constant 1.55 kgCmol⁻¹ leucine - carbon factor (Kirchman, 1993).

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Bouvier, T., Del Giorgio, P., and Gasol, J.: A comparative study of the cytometric characteristics of high and low nucleic acid bacteriaoplankton cells from different aquatic ecosystems. *Environmental Microbiology*, 9, 2050-2066, 2007.

Page 191, line 17, duplicate samples are hardly enough for leucine incubations. Need at least 3 or 4 replicates, and 2 killed controls.

Two to four replicates are suggested in the original paper by Kirchman (1993). Two replicates and one blank is generally what we use here and have used in other papers of our group reporting BP data (e.g. Van Wambeke et al, 2000, 2002, 2008, 2009, Christaki et al. 1999, 2002, 2008 and many others...). We recognize that with the centrifuge method, most authors use triplicates and one blank which has the advantage to potentially discard some extreme values. However, for the routine measurements in open MS during the BOUM cruise, two duplicate and one blank were sufficient for the following reasons: the blank was always measured and was much higher than the signal (6.8% of the signal on average). The mean variation between 2 duplicates (absolute difference divided by 2) of BP measured between 0 and 150m depth (the BOUM data presented in this study) was only 6.3%. We believe our estimates to be robust.

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Page 192, line 7. Give some details of the drifting rig. How was it drifting? Did it have a submerged structure to integrate the current in say the upper 10 m?

The drifting rig simply served to incubate bottles at different depths in the water column. The device has been used in numerous previous French cruises by us and other authors in the Mediterranean and elsewhere. We specify now that (section 2.4, paragraph 1)

The rig was equipped with a line of buoys on the surface to counterbalance and equilibrate the weight in order to maintain incubation bottles at the desired depths. Rates were measured at up to 9 depths of decreasing irradiance (75, 55, 35, 20, 10, 7, 3, 1, 0.1 %), to encompass the euphotic zone. The 0% irradiance samples corresponded to 130, 160 and 147 m depth for A, B and C respectively. The incubation depths were determined before every deployment based on the irradiance depth profile measured with a PAR sensor (Photosynthetically Active Radiation)

Page 195, line 10. What is ddl? degrees of freedom (df)? Why is it of 101 instead of the total 111?

yes it is degree of freedom and is corrected into df

101 is correct: df is n-2 so here (31+72)-2=103-2=101

31 for the W basin and 72 for the E basin, 111 is the whole data set for ciliates.

Page 198, line 12, Here 3 basins are invoked while the authors group their station in two basins and even discard some stations that would probably fall in the "central basin"

We do not 'group' our stations in east and west. We only discarded the central basin stations to see if the relationship between chl-ciliates showed the same pattern as in previous studies where only these 2 basins were considered (e.g. Dolan et al. 1999, Pitta et al 2001, reviewed in Siokou et al. 2010, p199-200 discussion section).

The text in section 3.1 now reads

Below, in order to facilitate comparison with previous studies which considered the contrast between the eastern and western Mediterranean, we will sometimes refer to E and W basins,

based on the simple geographical criterion employed by Longhurst (1998). Based on this division W and E stations are considered from 27 to 19 and from 13 to C, respectively. St. 17 and 15, situated in the Strait of Sicily are not included in the comparisons E-W, but these stations are included in the overall correlations between biological variables.

Page 199, line 5. Of what "relationship"? One should not have to read Gasol (1994) to guess what relationship the authors refer to.

We have cut the Gasol reference, so the text in 4.1, paragraph 2 now reads:

The significant relationship between log-log HNF and HBA values ($n=153$, $r^2=0.29$, $p<0.0001$) and the relative invariability of bacterial numbers together suggest that bacterial production was tightly matched by bacterial mortality.

Technical corrections

Page 188, line 12. The sentence does not make sense; something is incorrect or missing. It probably is "...Mediterranean, where the..."

It now reads (introduction)

Thus, microbial heterotrophic activity is an important energy pathway in the planktonic food web and in particular in the eastern Mediterranean, where most of the organic carbon produced is consumed and respired (Regaudie-de-Gioux et al., 2009).

Page 189, lines 21-23. The sentence needs to be properly written.

It now reads (section 2.1)

The BOUM cruise took place during the summer of 2008 (June 16-July 20, 2008). Using the French Research Vessel I'Atalante, a 3000 km transect was surveyed from the western part of the Mediterranean Sea to the Eratosthenes sea mount in the eastern part along a longitudinal transect from the Levantine basin (34° E) to the Western basin (5° E, Fig. 1). Along this transect, two types of stations were sampled: the "short duration stations" (2-3 h occupation) and "long duration sites" (4 days occupation). Except St. 27 and 17 located on the continental slope, the stations were situated in the open sea (Fig. 1).

Page 192, lines 15 and 24, uniformize the subscript of Pp.

done

Page 194, line 5. "The fluorescence maximum depth increased from 30 m...". But attention, there is a 120 m DCM at B!!!

It now reads (section 3.1)

The classical W-E gradient of deepening of the DCM was observed; the DCM was, in general, over 30 m deeper in the east compared to the west (Fig. 3a). Mean Chl-a values in the upper 150 m layer were very low, 0.1- 0.2 $\mu\text{g L}^{-1}$ except at the two NW stations where they were slightly higher (St. 25, 27, Table 1).

Page 194, line 25, Bacterial production cannot be found on Fig 2.

It is now added – see new Fig. 2

Page 197, line 16-18. The sentence of the comparison between dates at a station and between stations should be clarified. Right now it is stated incorrectly and the reader cannot make sense of it.

It now reads

"At sites A and B the two Pp integrated values measured the 1st and the 3rd day of occupation were similar (156 vs. 164 and 195 vs. 187 mg C m⁻² d⁻¹, respectively), while they showed some variability at site C (137 vs. 191 mg C m⁻² d⁻¹)."

Page 197, line 19-21, which one is x and which one y?

It is generally understood that Primary Production (PP) is the independent variable since dissolved organic carbon (DOC) generated from primary production by a variety of means is taken up by heterotrophic prokaryotes (Bacterial Production) and used for their growth and metabolism.

Page 202, line 26, DYFAMED in capital letters
done

Page 210, Longitude and Latitude headings have to be interchanged.

Page 210. I cannot make out Lat and Long data: 4° 93,050' does not exist as there are only 60 minutes in a degree. I guess it is 4.93050° and so on.

Interchanged and corrected

Page 212. Remove colon. "...(Pp_{total}) during the first (A1, B1 and C1) and third (A3, B3 and C3) day of site occupation."

Corrected

Page 214. I cannot read the axis values and color scales of the different plots at 100%. If the plots have to be printed in grayscale, some colors overlap in grays. Also, a linear scale for some of the variables which need to be multiplied by a power of 10 and or otherwise when the scale does not reach 0, makes it almost impossible to get a clear picture in areas of low concentration. Log scales may be better.

Fig. 2 has been split in two. New figures 2 and 3 have larger scales for numbers when inserted along isolines in the graph, so that even printed W&B versions of the figure could be read properly. Use of a log scales did not improve readability. The graphs will be produced in colour that could be always enlarged and viewed on a computer screen. On all the color codes the zero have been added.

Page 215. The legend reads "temperature and salinity" but the plots only show salinity. There was a mistake in the legend. Temperature is deleted.

Page 219. The legend of the figure reads A3, B3, C3 but the figure axis shows A2, B2, C2.
Corrected

REVIEWER 2

Review of "The impact of anticyclonic mesoscale structures on microbial food webs in the Mediterranean Sea" by Christaki et al.

General comments

Christaki et al. present an interesting data set of the abundance and activity of the major members of the heterotrophic microbial community along a longitudinal transect carried out in the Mediterranean Sea in summer 2008. The main goals of this study were 1) to provide a general description of the heterotrophic compartment along a W-E transect, and 2) to characterize the relative contribution of the microbial food web to the cycling of organic

matter in three distant anticyclonic mesoscale eddies. My main concern about the manuscript is that so far it fails to accomplish the second goal, being the main reasons for that:

1) Identification of mesoscale features is not supported by hydrographic information. Mesoscale features are very dynamic and exhibit important temporal and spatial variability. Physical characterization is not trivial and requires a combination of different approaches including remote sensing techniques and different types of hydrographic data. When only CTD data is available, at least two perpendicular transects are needed to identify mesoscale eddies. High spatial resolution data is also required in order to characterize stations that are under the influence of mesoscale dynamic and those stations that can be considered as background reference stations. The first step in order to study the effect of mesoscale features in the microbial community is to carefully identify stations under the influence of mesoscale dynamics and background reference stations. In this study only single salinity profiles of same stations that are considered to be inside and outside of the mesoscale features are presented. The salinity profile at station A shows that the center of this mesoscale feature is characterized by lower salinity compared to the reference station (st 21), whereas mesoscale features centered at stations B and C were characterized by higher salinity compared to the reference stations. The paper does not provide to the reader with any evidence that stations A, B and C were located inside of mesoscale features. A detailed explanation of how the mesoscale features were identified is the first step needed to accomplish the second goal. See response to 'Issues raised by both reviewers 1 & 2' above

2) The proposed hypothesis is very vague.

The authors present as a vague hypothesis that the impact of eddies will be recognizable within the W-E gradient of oligotrophy. However they do not specify which type of impact it should be expected. This point is related to the previous one as a complete description of the type of mesoscale features, their impact in the surface layer and the expected effect in the microbial communities is needed. Moreover, the authors' concern about the manuscript is that so far it fails to accomplish the second goal, being the main reasons for that:

1) Identification of mesoscale features is not supported by hydrographic information. Mesoscale features are very dynamic and exhibit important temporal and spatial variability. Physical characterization is not trivial and requires a combination of different approaches including remote sensing techniques and different types of hydrographic data. When only CTD data is available, at least two perpendicular transects are needed to identify mesoscale eddies. High spatial resolution data is also required in order to characterize stations that are under the influence of mesoscale dynamic and those stations that can be considered as background reference stations. The first step in order to study the effect of mesoscale features in the microbial community is to carefully identify stations under the influence of mesoscale dynamics and background reference stations. In this study only single salinity profiles of same stations that are considered to be inside and outside of the mesoscale features are presented. The salinity profile at station A shows that the center of this mesoscale feature is characterized by lower salinity compared to the reference station (st 21), whereas mesoscale features centered at stations B and C were characterized by higher salinity compared to the reference stations. The paper does not provide to the reader with any evidence that stations A, B and C were located inside of mesoscale features. A detailed explanation of how the mesoscale features were identified is the first step needed to accomplish the second goal.

See response to 'Issues raised by both reviewers 1 & 2' above

2) [The proposed hypothesis is very vague – the same comment appears twice –below – single response below.](#)

-The authors present as a vague hypothesis that the impact of eddies will be recognizable within the W-E gradient of oligotrophy. However they do not specify which type of impact it should be expected. This point is related to the previous one as a complete description of the type of mesoscale features, their impact in the surface layer and the expected effect in the microbial communities is needed. Moreover, the authors conclude that mesoscale features were characterized by a reduction in different heterotrophic compartments compared to the background. The paper is very descriptive and no explanation is given for this observation that disagrees with several previous publications reporting an increase of heterotrophic activity inside of anticyclonic mesoscale features (Ewart, 2008, DSR II; Baltar et al., 2010, ISME; Mouriño et al., 2009, DSR I).

- Page 200, lines 13-19 "Our hypothesis was that the impact of eddies would be recognizable within the broader W-E gradient of oligotrophy. The 3 eddies were indeed associated with the lowest values for different metrics of the heterotrophic compartments of the microbial food web. This was particularly pronounced for ciliates (both heterotrophs and mixotrophs) which are the link between microbial food web and the higher trophic levels."

This hypothesis needs a justification. Which is the reason to expect a decrease in the heterotrophic activity inside these anticyclonic eddies?

The hypothesis was that the position of eddies would be relevant for the biomasses and rates present. Since C was in the East we would expect to find less biomass and production than in the A which was in the West. In the new version of the paper we have reconsidered the objectives relative to the eddies. The major contribution of our paper is that for the first time we provided information on the metabolic state of open Mediterranean waters, focussing in 3 well defined anticyclonic eddies situated in 3 distinct basins. In fact, the global dataset of respiration, when compared to that of ¹⁴C-based PP, is about 1% (Williams and DelGiorgio, 2005).

See revised 4.2 section

Regarding the studies mentioned by the reviewer, in none of these studies was community respiration measured so our results on the metabolic state can not be in disagreement with them. Furthermore, we believe the reviewer is mistaken with regard to our report being in disagreement with previous observations.

A large variety of relative activity rates have been reported with regard to cyclonic compared to anti-cyclonic eddies as well as eddies of different ages. The Baltar et al. (2010) paper reports unusual results as both cyclonic eddies and anti-cyclonic (both 'young & 'mature') eddies are all described as showing high prokaryote activities both per cell and as bulk rates, interestingly with no differences in community composition in surface layer communities. Most commonly, increases in heterotrophic activity characterize cyclonic eddies, immature 'cold core' eddies. More importantly however, very few studies have attempted to estimate overall metabolism through comparing phytoplankton production, bacterial production and community respiration.

Thus, our goal was not simply to compare prokaryotes inside and outside the eddies but rather to gauge their activity relative to the other components of the planktonic food web. The eddies investigated were located along a longitudinal axis corresponding with a gradient of increasing oligotrophy. We hypothesized that the eddies would also display a gradient of overall community heterotrophy analogous to the general west to east gradient.

[Role of mesoscale feature – similar comment appears several times - single response below.](#)

-The presented data set does not allow to discuss the role of mesoscale features on microbial activity fluxes.

-Main fluxes reporting microbial activity are only presented inside the mesoscale features (Figure 7). As no information is provided about the magnitude of these variables in stations not influenced by mesoscale dynamics it is not possible to distinguish the effect of these features over the background.

-Page 189, lines 1-4 “One of the central ideas of the BOUM cruise (Biogeochemistry from Oligotrophic to the Ultra-oligotrophic Mediterranean) in summer 2008, was that, besides the general aspect of oligotrophy in the Mediterranean, the mesoscale discontinuities likely influence biological processes”. Specify the expected impact.

-Page 197: Biological fluxes in the 3 anticyclonic eddies. Note that as biological fluxes are only shown inside the mesoscale features it is not possible to discuss the effect of eddies in these variables.

Abstract: The abstract should be modified to address the comments raised above. PPp is not defined.

The objectives have been modified, see last paragraph of Introduction.

We do not focus on the ‘effects’ or ‘impact of eddies’ We just discuss stocks and biological fluxes estimated for the 3 oligotrophic eddies situated in the 3 Mediterranean basins obtained with IN SITU measurements.

PP is now defined; the abstract has been modified accordingly.

[Integrations and table 1](#) - similar comment appears several times -- single response below.

- Page 195, lines 12-13: the contribution of mixotrophs to ciliate biomass is not shown. I recommend to include this information (based on depth integrated values in table 1)

- Page 200, lines 2-3 "Overall, mixotrophs showed a high variability in their contribution in total ciliate biomass (Table 2)..."

-The authors may consider to include a new column in table 2 showing mixotrophs contribution to total ciliate biomass.

The contribution of mixotrophs is treated in some detail. They are presented in Table 2, Fig. 2e, and Fig. 8, and the text. We hesitate to devote any more data presentation or further discussion on mixotrophs in the interests of maintaining a balanced presentation.

Page 195, lines 25 “All the heterotrophic parameters recorded showed lower values: : :”

Is this true for HNF? Here and for the other variable I recommend to make the comparison based on depth-integrated values.

Page 196, lines 25-26 “Finally, ciliates showed in both cases maximal abundance just above the DCM and were about 2 more abundant at St. 13 compared to inside the eddy.” This pattern is not very clear inside the eddy. I recommend to compare depth integrated values.

To resolve this problem (repeatedly mentioned by the reviewer) the last figure is now presented earlier as fig. 5. Note that we also added the BP integrated values.

Comments

Title: The title is too ambitious. So far the paper does not correctly address the impact. The authors present as a vague hypothesis that the impact of eddies will be recognizable within the W-E gradient of oligotrophy. However they do not specify which type of impact it should be expected. This point is related to the previous one as a complete description of the type of mesoscale features, their impact in the surface layer and the expected effect in the microbial communities is needed. Moreover, the authors conclude that mesoscale features were characterized by a reduction in different heterotrophic compartments compared to the

background. The paper is very descriptive and no explanation is given for this observation that disagrees with several previous publications reporting an increase of heterotrophic activity inside of anticyclonic mesoscale features (Ewart, 2008, DSR II; Baltar et al., 2010, ISME; Mouriño et al., 2009, DSR I).

- Page 200, lines 13-19 "Our hypothesis was that the impact of eddies would be recognizable within the broader W-E gradient of oligotrophy. The 3 eddies were indeed associated with the lowest values for different metrics of the heterotrophic compartments of the microbial food web. This was particularly pronounced for ciliates (both heterotrophs and mixotrophs) which are the link between microbial food web and the higher trophic levels."

This hypothesis needs a justification. Which is the reason to expect a decrease in the heterotrophic activity inside these anticyclonic eddies?

of mesoscale eddies. Also it should clarify that the paper only deals with the heterotrophic component of the microbial community.

The title has been changed. We note though, that our paper also deals with the metabolic status of open Med waters in summer.

Page 188, line 1: Krom et al., 1993 (inconsistent with the reference list, Krom et al., 1991)

Corrected

Page 189, lines 11-13 "Our hypothesis was that the impact of the eddies will be recognizable within the broader W-E gradient of oligotrophy." Again specify the expected impact.

We expected that the eddies would show differences in terms of biomass and production compared to outside stations. In this paper we will refer only to biomass and bacterial production since the measurements of community production and respiration were only made in the eddies (see end of new introduction).

Page 189, line 23: temperature and oxygen data are not presented in this paper.

We added that 'temperature and oxygen data are not used in this paper' –

Page 190, line 23: Porter and Feig (1980) not in the references list.

Added

Page 192, lines 21-22 "They sampled sea water for such measurements at selected depths from a CTD the CTD cast used for the 24 h-long: : ." modified to "They sampled sea water for such measurements at selected depths from the CTD cast used for the 24 h-long: : ."

Added

Page 194, line 7 "Mean Chl-a values in the upper 150m layer were very low: : ." For this and all the other variables presented in table 1 I recommend to use depth integrated values in the photic zone instead of mean values in the upper 150 m.

The chl figure (new Fig. 2a) is now added, to give a better idea of what we are talking about. Primary Production is integrated down to the depth of % 0.1 light level. We were interested in heterotrophic biomass and production below the euphotic layer. We do not want to lose this information and we want have comparable profiles along the transect. In fact we would like to have data even deeper, down to 250 m for heterotrophs but we had to make a compromise with our means.

Page 194, lines 17-19 "VLP was relatable to Chl-a concentration (n=116, r2 = 0.293, p<0.0001) but a tighter relationship existed between VLP and heterotrophic bacterial abundances (HBA) (n=116, r2=0.505, p<0.0001)." For this and all the variables specify

if the correlation was built with volumetric or mean values. The authors may consider to include a new table reporting all the correlations coefficients between all the variables and why some of them were converted to log distributions.

Volumetric was specified just above, it is now repeated in the sentence.

We do not report 'correlations coefficients' (r) but 'determination coefficients' (r^2), which quantifies the % of variance of the dependent variable explained by the independent variable. The log transformation is usually used for variables which vary over several orders of magnitude, and/or for variables of different orders. This also allows comparison with previous studies (for example logPP vs logBP).

Page 194, line 25: Fig 2c does not correspond to bacterial production.

BP is now added

Page 195, lines 5-6 "Highest ciliate abundances were recorded at the DCM level or just above it." Is this true in the western side of the transect? I recommend to overlap chlorophyll distributions in the different panels if figure 2.

Yes it is. As already stated above, Chl is now added

Page 195, line 12: does Fig. 2e refer to Fig. 2f?

corrected

Page 195, line 14: does Fig. 2d refer to Fig. 2e?

corrected

Page 195, lines 21-22 "At site A the bottom depth was 2800 m, the anticyclonic eddy was detectable down to 800m and the core depth of the eddy was 100–250m (Fig. 3a)." For this and the other eddies provide information to support the identification and vertical extent of the eddy core.

See response to major comments 1 and 2 above (i.e., Section 2.1 in revised ms).

Page 195, line 23: here and through the manuscript, what does smooth mean?

Page 196, line 1: Explain what "irregularities" means.

Page 196, line 12 "flat nutrient profiles", does flat mean lower?

We thought the terms 'smooth' and 'flat' to be unambiguous. Smooth is generally defined as 'without bumps, peaks or irregularities' and flat is understood to describe something invariant or featureless. With regard to figures, flat and smooth were used to describe vertical profiles.

Although we are not sure what the problem is here these words, they were replaced by:

"showing little or no increases in concentration with depth, showed less depth-related variability, showed several peaks, nearly featureless, "

Page 196, lines 14-15 " : : and generally lower values and smoother profiles inside the eddy 1 (Fig. 5a–f)." Figure 5 does not show this pattern for virus and HNF.

Changed into .."generally lower values for nutrients, BP, ciliates and less pronounced maxima for fluorescence"

Page 198, lines 9-10 "Our study is the first, to our knowledge, to encompass all the major components of the microbial food web..."

Indicate heterotrophic microbial food web.

Indicated

Page 199, lines 19-21 "The mixotrophic/autotrophic ciliate *Myrionecta rubra* was pooled with taxa of mixotrophic ciliates (*Tontonia* spp., *Laboea strobila*, *Strombidium acutum*, *Strombidium capitatum* and *Strombidium conicum*)."

Indicate that these data are not shown.

Now indicated that 'Detailed specific composition of ciliates was not attempted.'

-Page 200, lines 13-19 "A question that arises is: Is the oligotrophy gradient detectable among eddies? The W-E gradient, although attenuated, was clearly recognizable among the 3 eddies and except for virus in site C, all stocks are higher at the stations outside the eddies (Fig. 8)." I have strong concerns that with the presented data set the authors can distinguish between the background oligotrophy W-E gradient and the eddy effect, as mesoscale features are not well characterized. Based on figure 8 bacteria stocks are higher in station C than station 11.

- Page 200, lines 27-28 "Overall, a W-E gradient among the eddies was generally recognizable in terms of heterotrophic biomass values but not in terms of production. The background pattern in terms of production is not shown."

-Page 201, lines 28-29 "Integrated BP was very similar at the 3 eddies ($33.5 \pm 6 \text{ mg C m}^{-2} \text{ d}^{-1}$). Where does this value come from?"

Response to the above 3 comments:

The mesoscale features were characterized by a group of physicists participating in BOUM cruise (see Issues raised by both reviewers &1 & 2).

Integrated BP is added in new fig. 5

$33.5 \pm 6 \text{ mg C m}^{-2} \text{ d}^{-1}$ was from repeated measurement over diel cycles in the eddies (Van Wambeke et al. this issue) it is now corrected with the value obtained from the PP casts shown on table 3 ($30.2 \pm 5.7 \text{ mg C m}^{-2} \text{ d}^{-1}$) Regarding the W-E gradient and eddies,

Section 4.2, has been reorganised

First part now reads:

A major aim of the BOUM cruise was to estimate biogeochemical fluxes in open Mediterranean waters during the period of summer water-column stratification. The biogeochemical and the stoichiometric characterisation of stations along the entire cruise transect showed the expected oligotrophic gradient from the western basin to the Levantine basins (Pujo-Pay et al, 2011). In our study we focussed on 3 well established anticyclonic eddies located within the west to east gradient. The centre of established anti-cyclonic eddies are known to be zones of nutrient depletion with low rates of biological activity compared to surrounding areas (e.g. Mourino-Carballido 2009). We found that the 3 eddies were indeed associated with low values for different metrics of the heterotrophic compartments of the microbial food web compared to the stations located outside the eddies. Metazooplankton biomass was also found to be lower inside the eddies compared to stations outside the eddies (Nowaczyk et al., 2011, this issue). We hypothesized that the general W-E gradient of oligotrophy would be detectable among the eddies. While the W-E gradient among the eddies was generally recognizable in terms of heterotrophic biomass (Fig. 5), the same was not true for fluxes. Means of integrated P_{pp} values were higher at site B (~190 mg C m⁻² d⁻¹) and about 15 % lower at sites A and C (~160 mg C m⁻² d⁻¹). Surface oxygen production roughly balanced by respiration. NCP was not significantly different from zero in all three sites ($4 \pm 15 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$) (Fig. 9) showing systems in metabolic balance (Williams, 1993).

Page 202, line 10 "Crombet et al., this issue"

Update this reference

updated

Page 202, lines 11-13 "A plausible explanation might be the presence of relatively elevated PO₄ values in the entire surface layer (0–150 m), generally 4 times higher than those at stations A and B, despite a deeper phosphocline at station C."

Were higher PO₄ values observed in more than one nutrient profiles carried out at station C?

Yes, they were repeated twice, Text now notes:

Page 202, lines 14-17 "However, we might argue that the elevated phytoplankton biomass and production observed at station C were related to those higher PO₄ concentrations, at an optimum depth (80–100 m) where there were enough NO₃ and light to sustain productivity and growth." Why were not the higher PP values observed in both production experiments?

We have no explanation for the variability found between the two station C experiments.

Page 202, lines 17-19 "Most probably, the bottom topography and vertical structure of the eddy at station C (warmer and shallower) may have played a critical role in structuring the above mentioned characteristics."

Explain how bottom topography and the vertical structure of the eddy are expected to play a role in the vertical distribution of primary production

We sought only to mention a possible link between nutrient concentrations and eddy structure/bottom topography. This should now be clearer:

"Most probably, the bottom topography and vertical structure of the eddy at station C (warmer and shallower) may have played a critical role in structuring the nutrient profiles (for full details on the physics, Moutin et al. this issue)"

Page 202, lines 28-29 "BR ranged from 0 to 36.4 mmolO₂ L⁻¹ d⁻¹ (Lemée et al., 2002; Navarro et al., 2004)."

When? Give more details about this study

The sentences have been modified

"In the NW Mediterranean Sea, at the open water DYFAMED site, the mean ratio BR/DCR over an annual cycle was 65% (Lemée et al., 2002), and at a coastal site. In another annual cycle study in oligotrophic coastal Mediterranean Sea waters, an average value of 52 % (range 41 to 85%) was recorded by Navarro et al. (2004). BR ranged from 0 to 36.4 mmol O₂ L⁻¹ d⁻¹ (Lemée et al., 2002; Navarro et al., 2004). "

Page 203, lines 16-19 "Our integrated GCP (28–75 mmolO₂ m⁻² d⁻¹) and DCR (39–58 mmolO₂ m⁻² d⁻¹) values fell within the range of previously recorded values except the ones reported by Regaudie-de Gioux et al. (2009), which were higher (mean GCP 118–196 mmolO₂ m⁻² d⁻¹)."

When and where? Give more details about the study by Regaudie-de Gioux et al. (2009)

We state that these authors reported data from Mediterranean transects and cite their observation that season variability is likely important

"With the exception of a seasonal study at a fixed station in the Ligurian Sea (Lemée et al., 2002) and a late-spring early summer cruise in the open Mediterranean Sea by Regaudie-de Gioux et al (2009),....."

Page 203, lines 23-24 "NCP varies with geographical, temporal, seasonal scales and is also strongly influenced by mesoscale variability (del Giorgio and Duarte 2002; Maixandean et al., 2005)."

Seasonal are temporal scales. Mesoscale involve temporal and spatial variability scales. Modify this sentence.

Sentence now reads:

"NCP varies with geographical, temporal, seasonal scales and is also strongly influenced by mesoscale features (del Giorgio and Duarte 2002; Maixandean et al., 2005)."

Page 203, lines 27-28 "Assuming then that heterotrophic bacterial respiration was 50% of DCR (see below),..." modify to "Assuming then that heterotrophic bacterial respiration was 50% of DCR (see above),..."

modified

Page 204, lines 1-3 "The net heterotrophy, although not statistically significant at site A, is in accordance with the BCD/PPTtotal ratio slightly higher than 1."

Is not the net heterotrophy at station A inconsistent with the W-E increase gradient in oligotrophic conditions?

The system is in metabolic balance. According to our data the 3 eddies showed equilibrium between GCP and DCR, and thus NCP is not statistically different from zero. The results are not inconsistent, since all the results show that station A is an oligotrophic site.

Page 204, lines 10-12 "Limited data on ciliate community composition suggested that Eddy microbial communities differed from those in adjacent stations outside the eddies."

This pattern is not clear at station C

Revise the conclusions section based on the comment above.

We refer to the community composition of station B – it is stated in the paper: With regard to species assemblages, the tintinnid ciliate community appeared distinct with 11 species detected in the eddy samples only 4 of which were found also at St. 13.

We think it is a quite interesting result, but it is indeed limited to station B. This sentence is now deleted from the conclusion.

References: Schlitzer, R.: Ocean Data View 4, <http://odv.awi.de>, 2009. (Schlitzer, 2010 in the text)

The algorithm reference is 2004 but the project has many updates, one can find now the 2011 version. To keep it simple it is now referenced as follows

Ocean Data View[®] (Schlitzer 2004) <http://odv.awi.de/>

Table 1: Consider to show depth-integrated values instead of mean values. Which is the justification for the 150 m limit? Why dont using the base of the photic zone?

We counted samples for heterotrophs down to 150 m which is always below the 1 % incident light (Z_e) We present the data we obtained in order to have comparable results at all [the](#) stations.

Table 2: I do not see the need for this table if table 1 shows depth-integrated values

Our choice for table 1 was to show minimum –maximum and mean values which are very relevant. We tried including integrated values but this made the table impossible to read.

Table 3: Include error from replicates

Profiles of PPp fluxes were measured twice at each site and we had 2 replicates and a control for the in situ incubations. The replicates of each depth are used to calculate the value of each depth, based on this we did the integrated values. There are no other replicates to add here.

Figure 2: Consider to include an overlapping plot showing chlorophyll distribution. Increase label size. Indicate station number.

Chl figure included. Fig. 2 split in two figures. Label increased. BP also added. Station are indicated with vertical dashed lines.

Figure 3: Temperature profiles are not included.

The word Temperature is deleted from the legend

**Figure 7: Modify labels A2, B2 and C2 to A3, B3 and C3.
modified**

Reviewer 3

Many of the presented results rely on the euphotic depth and determination of the light levels, but no information at all is given how those were determined. This needs to be changed in the revision.

We added a sentence

The incubation depths were determined before every deployment based on the irradiance depth profile measured with a PAR sensor (Photosynthetically Active Radiation). Some minor points are listed below:

Abstract, line 4 before end PPp used but not explained

Ok, indicated

P188, last line: Cyprus-Eddy

Ok, - added

P190: line 11: until

Until analysis

analysis Line 18: FCM used but not explained

ok, indicated

Page 191: factor of 1.5kgC per mole really as high as this? We checked during BOUM cruise that the dilution factor was close to 1 when using 20 nM leucine as final concentration. Hence the dilution factor was stable and equal to 1 and this is why we used a constant 1.55 kgCmol⁻¹ leucine - carbon factor (Kirchman, 1993).

Reference

Kirchman, D.L., 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In: Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole, J.J. (Eds.), Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton, pp. 509–512.

P192, line 4: To explain what that I believe starting point, but maybe I'm wrong L21-24: Sentence needs to be re-formulated, as is not clear

Text now reads

"To determine quantity of added tracer, 250 µl of sample was taken at random from 3 bottles and stored with 250 µl of ethanolamine for later analysis. For time zero determinations, three samples were filtered immediately after inoculation."

P193: add blank in front of 145 m

OK

P194 and following: do not give the corr. coefficients with 3 or 4 decimal positions, two will do as well

OK, corrected

P195, line 24: observed at the

OK

P196, line 3: concentrations being higher

ok

line 6: with three times more line

ok

7: ciliates were more line

Sorry can not see the problem

8:, contributing approximately line

ok

26: were about two times

ok

P197, line 18: values at site B

Sentence changed

line 29: distinguishable only between...

can not find line 29

Page 198, line 7: MS and following pages I guess it is Mediterranean Sea?

MS was used a number of times but not systematically for simplicity. It is now replaced with Mediterranean Sea

line 16: on viruses

ok

Page 199, line 1: two times

OK

Page 201, line 21: lower at site A

OK

Page 202, line 1 Cyprus-eddy

line 6: than those at station

OK

Page 204, line 1: at site A

OK

Figure 2: in this figure you are using pressure / dbar but in others depth /m make it consistent please or explain why pressure can be used for depth

changed in meters (now fig.2 and 3)

Figure 3 only salinity is presented but the caption refers to temperature as well

Word Temperature deleted

Figure 8 caption: take all the abbreviations in the brackets away, no need for them as they are not used in figure itself

done